REPORTS

Statewide Study of Diagnostic Agreement in Breast Pathology

*Wendy A. Wells, Patricia A. Carney, M. Scottie Eliassen, Anna N. Tosteson, E. Robert Greenberg**

Background: **This study assessed the degree of diagnostic agreement among community-based general pathologists reading slides of representative breast tissue specimens and tested whether diagnostic variability is associated with type of breast specimen (e.g., core needle or excisional biopsy) or slide quality.** ***Methods:*** **Twenty-six of the 44 eligible pathologists working at community-based pathology practices in New Hampshire participated. Each pathologist evaluated slides of breast tissue obtained from 30 case subjects randomly selected from a statewide breast pathology database. The diagnostic categories used were benign, benign with atypia, noninvasive malignant, and invasive malignant. The levels of agreement (i.e., kappa coefficients) for the diagnoses were assessed.** ***Results:*** **Agreement was high among pathologists for assignment of diagnostic category (kappa coefficient = 0.71) and was nearly perfect for their selection of benign versus malignant categories (kappa coefficient = 0.95). There was less agreement for the categories of noninvasive malignant and benign with atypia (kappa coefficients of 0.59 and 0.22, respectively). There was no apparent relationship between levels of diagnostic agreement and specimen type or perceived slide quality.** ***Conclusions:*** **Diagnostic agreement for breast tissue specimens is high overall among community-based pathologists, but clinically relevant disagreements may occur in the assessment of noninvasive malignant diagnoses. The establishment of reread policies for certain diagnostic categories may reduce the possibility that diagnostic misclassification will lead to overtreatment or undertreatment. The high degree of diagnostic reproducibility for invasive cancerous lesions of the breast suggests that it is unnecessary for a central review of these lesions in national cancer trials. [J Natl Cancer Inst 1998;90:142–5]**

The frequency of diagnosis of breast cancer has increased markedly over the past 2 decades, particularly for noninvasive ductal carcinoma *in situ (1,2).* Much of this increase results from greater use of high-quality mammography and more frequent biopsy of suspicious findings. Previous studies *(3,4)* have found relatively poor agreement among pathologists in their diagnostic assessments of breast disease, but these studies have largely used pathologists in academic centers with a special interest in breast pathology, and the slides reviewed were from cases with challenging histologic features. There is scant information on the reproducibility of diagnoses provided by community-based pathologists *(5–7),* and no data have been published from a representative mix of biopsy specimens interpreted by pathologists in the United States. This report describes the degree of interobserver agreement for breast diagnoses among community-based general pathologists in New Hampshire.

Methods

The study was approved by an institutional committee for the protection of human subjects and endorsed by the New Hampshire Society of Pathologists. We sent recruitment letters and information detailing the proposed study and the lead investigator (W. A. Wells) met with each of the 44 eligible pathologists in New Hampshire. To be eligible to participate, a pathologist must have been actively practicing general surgical pathology in New Hampshire, have regularly evaluated breast tissue, and have reported no plans to retire or relocate within the study period. Each participant returned a signed consent form.

Forty-four pathologists met the criteria for eligibility, and 35 (80%) of these pathologists—representing 14 (82%) of the state's 17 hospitals with laboratories that process breast tissue specimens—agreed to submit breast pathology reports for all biopsied and excised breast tissue beginning in January 1996. Six pathologists from the only academic center in the state were also included. Data on specimen type (e.g., core biopsy or excisional biopsy) and diagnosis were entered into a central database. Pathologists also provided information on demographic/practice characteristics, usual content of breast pathology reports, and tissue processing methods.

After 3 months of data collection, the pathology database held information on 502 biopsy specimens. After stratifying the cases in the database by diagnosis, a random number table was used to select 30 case subjects with diagnoses representative of the distribution of all diagnoses in the database. We asked pathologists who had submitted the selected reports to submit four recut tissue slices of a representative block from the case. Each recut specimen, from a formalin-fixed, paraffin-embedded tissue block, was 4-μm thick and was stained with hematoxylin–eosin under standard conditions. The recut specimens were reviewed (by W. A. Wells) to ensure that the same histopathologic material was present on each recut tissue slice. The slides were masked and organized into four complete sets, each mailed according to a structured rotation schedule so that each pathologist read one set of 30 slides. Of the selected slides, nine were derived from image-guided core biopsy specimens (stereotactic or ultrasound guided) and 21 from excisional biopsy and mastectomy specimens.

All participating pathologists used a standard reporting sheet to record their interpretations of each slide in the circulated set. Summarized categories of diagnosis were: benign, benign with atypia, noninvasive malignant, and invasive malignant. The pathologists also evaluated each slide for processing, staining, and sectioning quality by categories of excellent, very good, satisfactory, and unsatisfactory. For slides with quality perceived to be less than very

**Affiliations of authors:* W. A. Wells (Department of Pathology), P. A. Carney, M. Scottie Eliassen, E. Robert Greenberg (Departments of Community and Family Medicine and Medicine, and The Norris Cotton Cancer Center), A. N. Tosteson (Department of Community and Family Medicine and The Center for Evaluative Clinical Sciences), Dartmouth Medical School, Hanover, NH.

Correspondence to: Wendy A. Wells, M.D., Department of Pathology HB 7999, Dartmouth-Hitchcock Medical Center, 1 Medical Center Drive, Lebanon, NH 03756. E-mail: Wendy.Wells@dartmouth.edu

See ''Notes'' following ''References.''

good, the participants were asked to detail the deficiency. Possibilities included inadequate tissue fixation, poor tissue processing (alcohol clearing and paraffin infiltration), section artifacts (thickness and wrinkles), and suboptimal staining. Participants were blinded to the original diagnosis and to each others' readings.

To assess diagnostic agreement, we computed a kappa statistic (i.e., coefficient) for the overall agreement in all four diagnostic categories and for comparisons between categories (e.g., benign cases versus malignant categories and noninvasive malignant cases versus all other categories). The kappa statistic estimates the level of agreement, after accounting for agreement that would be expected by chance alone. Kappa statistics less than 0.4 represent fair to poor agreement, values of 0.4 to 0.8 represent moderate to good agreement, and values over 0.8 represent excellent agreement *(8).* The impact of slide quality and sample source was also examined in subgroup analyses.

Continuing Medical Education credits were awarded to all pathologists completing the project, and each was sent a report comparing his/her individual interpretations with the statewide aggregate results. The results were presented at the annual meeting of the New Hampshire Society of Pathologists.

Results

Twenty-six (74%) of the 35 pathologists who submitted reports to the database took part in the slide review and contributed data to the current analyses. The characteristics of the 26 participants differed little from those of the 17 eligible nonparticipating pathologists (Table 1). Of the nine who did not provide data for the analyses, one (W. A. Wells) was ineligible (had viewed the slides during the selection process), three were excluded because they read the study slides as a group, and five chose not to participate in this portion of the project.

We received a total of 775 review diagnoses from the 26 participants who nearly all provided a diagnosis for each of the 30 slides. Five diagnosis review forms were left entirely blank, one each by five pathologists. The distribution of diagnoses for the study slides [489 (63%) benign, 47 (6%) benign with atypia, 66 (9%) noninvasive malignant, and 173 (22%) invasive malignant] was comparable to the distribution of diagnoses reported to the breast pathology database [330 (66%) benign, 18 (4%) benign with atypia, 28 (6%) noninvasive malignant, and 122 (24%) invasive malignant] at the time the random sample of 30 cases (representing 30 patients) was chosen.

There was a clear consensus on the diagnosis for almost every case, with complete agreement for 11 (37%) of the 30 cases (Table 2). For differentiation between benign and malignant categories, there was complete agreement for 22 (73%) of the cases. Clinically relevant diagnostic variations were observed in eight (27%) cases (N, O, P, Q, S, T, U, and V), with discrepancies in benign versus malignant diagnoses by one pathologist. For two of these cases (N and P), the majority diagnosis was benign with one diagnosis of invasive malignant. For three cases (X, Y, and Z), there was substantial disagreement between noninvasive malignant and invasive malignant. For six (20%) cases (H–M), the majority diagnosis was benign, but one pathologist made a diagnosis of benign with atypia. For these six cases, as well as for cases N, O, P, Q, S, T, U, and V, identification of the one pathologist who recorded a discordant diagnosis compared with all of the other pathologists revealed a different person in every case.

The kappa coefficient confirmed a high level of agreement for assignment of diagnostic category (kappa coefficient = 0.71) and near perfect agreement for the distinction between the two benign versus the two malignant categories (kappa coefficient = 0.95). Less reproducible diagnostic categories, compared with others, were the benign with atypia and noninvasive malignant, with kappa coefficients of 0.22 and 0.59, respectively (Table 3).

Only 30% of the participants indicated that they routinely review core biopsy specimens in their daily practice. However, the kappa coefficient for the nine image-guided core biopsy specimens was 0.85 overall and 0.98 for distinguishing between the benign and malignant categories. These figures were only slightly lower for the noncore biopsy specimens (0.60 and 0.85, respectively). Kappa coefficients for distinguishing between diagnoses of noninvasive cancer versus the other categories were 0.57 and 0.60 for the core and noncore specimens, respectively. The recognition of histologic special type invasive tumors (lobular and colloid) in both the core and noncore specimens was excellent.

For slides where reviewers rated the quality lower than very good, the most commonly cited deficiencies were fixation and staining quality. However, reduced quality did not seem to affect diagnostic agreement. The kappa coefficient for slides interpreted as of high quality (rated by $\geqslant$75% of participants as excellent, very good, or satisfactory) was 0.64. For slides classified as unsatisfactory or rated by greater than or equal to 25% of reviewers as only satisfactory, the kappa coefficient was 0.69. The twelve pathologists classifying 17 slides as unsatisfactory, attributed the poor quality roughly equally to fixation, staining, sectioning, and processing. No single laboratory was responsible for consistently substandard slide quality.

Nineteen (66%) of 29 pathologists completed our survey about breast pathology reread procedures (defined as a second pathologist giving an independent evaluation of all or some breast pathology cases). Of these, 16% reported rereading all breast tissue cases (benign and malignant). An additional 37% reported rereading all malignant, benign with atypia, and noninvasive malignant cases. Rereading of specimens originally diagnosed as benign with atypia or noninvasive malignant was reported for 21% and 26% of cases, respectively.

Discussion

This study indicates a high level of diagnostic agreement for the type of breast pathology material routinely reviewed in practice by community pathologists in New Hampshire. None of these pathologists has a special expertise in breast pathology.

There were high levels of agreement (i.e., high kappa coefficients) for all four

Table 1. Characteristics of eligible participating and nonparticipating pathologists*

Characteristic	Eligible nonparticipants (n = 17)	Participants (n = 26)
Median age in y (range)	53 (35–65)	47 (36–65)
Median time in practice in y (range)	15 (4–20)	16 (2–37)
% Male	100	69

*Note: one pathologist (W. A. Wells) is excluded from this table (ineligible to participate in slide read, but contributes reports to the database).

Table 2. Distribution of diagnoses (n) by slide for the 30 representative cases

Slide	Benign (n)	Benign with atypia (n)	Noninvasive malignant (n)	Invasive Malignant (n)
A	26	0	0	0
B	26	0	0	0
C	26	0	0	0
D	26	0	0	0
E	26	0	0	0
F	26	0	0	0
G	24	0	0	0
H	25	1	0	0
I	25	1	0	0
J	25	1	0	0
K	25	1	0	0
L	25	1	0	0
M	25	1	0	0
N	24	1	0	1
O	23	1	1	0
P	23	1	0	1
Q	22	3	1	0
R	22	4	0	0
S	19	6	1	0
T	13	12	1	0
U	13	12	1	0
V	0	1	25	0
W	0	0	1	25
X	0	0	6	20
Y	0	0	13	12
Z	0	0	16	10
AA	0	0	0	26
BB	0	0	0	26
CC	0	0	0	26
DD	0	0	0	26

diagnostic categories, but particularly for distinction between the benign and malignant categories, between the invasive malignant category and all other categories, and between the benign (without atypia) category and all other categories. This is a higher level of agreement than was reported in a prior study of diagnostic reproducibility of proliferative breast lesions *(4)*. The slides reviewed in that study *(4)* were selected to include a high proportion of controversial and difficult borderline lesions; our slides comprised a representative sample of the diagnostic categories seen routinely in a general pathology practice. The participants in the prior study also used mutually agreed on diagnostic criteria while our participants followed their individual criteria for diagnosis within a standardized checklist.

Despite the excellent agreement overall, there are situations when anything less than perfect agreement may be clinically unacceptable. A diagnosis of cancer, when none is present, may result in unnecessary therapy and concern. Similarly, misdiagnosing cancer as a benign condition would result in needed therapy not being received. In this study, such critical disagreements occurred primarily in the differentiation between diagnoses of benign with atypia and noninvasive malignant. In most institutions, a woman whose breast biopsy diagnosis is benign with atypia receives follow-up surveillance and no treatment, whereas a noninvasive malignant diagnosis warrants at least surgical excision and often more extensive treatment *(2)*. Among the 30 reviewed cases in our study, five (8%) of 66 diagnoses of noninvasive malignant (cases O, Q, S, T, and U) represent instances where the consensus opinion of the other pathologists was that no cancer was present. In seven instances of a noninvasive malignant diagnosis (cases W and X), most pathologists had diagnosed invasive cancer; in two cases (Y and Z), pathologists were approximately equally divided between invasive and noninvasive assessments. There were two instances of a diagnosis of invasive malignant for which the consensus opinion was no cancer (cases N and P), and one instance of a diagnosis of no cancer (benign with atypia, case V) where the consensus opinion was that cancer (noninvasive) was present. Most pathologists in our state have told us they confer with their colleagues in difficult diagnostic breast cases; therefore, these disagreements, usually representing the divergent view of one pathologist, would almost certainly have been exposed by a second evaluation. Disagreements might also be reduced through use of standardized diagnostic criteria for the differentiation between benign with atypia and noninvasive malignant categories *(4)*. Since only 30% of the pathologists in New Hampshire evaluate image-guided core biopsy specimens, the exceptional diagnostic agreement for these specimens throughout the state suggests that fears of a prolonged learning curve for the evaluation of such biopsies by pathologists when a stereotactic or ultrasound-guided service is introduced are unfounded.

Our study is one of few that have focused on the diagnostic reproducibility of routinely practicing pathologists without a special interest or expertise in diagnostic breast pathology. The most comprehensive study evaluating consistency of histopathologic reporting was carried out

Table 3. Kappa coefficients* for randomly selected slides in the four diagostic categories

Diagnostic category comparisons	All slides (n = 30)	Image-guided core biopsy specimen slides (n = 9)	Excisional or mastectomy specimen slides (n = 21)
Benign versus malignant†	0.95	0.98	0.94
Benign without atypia versus all other categories	0.79	0.94	0.73
Benign with atypia versus all other categories	0.22	—‡	0.21
Noninvasive malignant versus all other categories	0.59	0.57	0.60
Invasive malignant versus all other categories	0.85	0.83	0.85

*There were 24 to 26 independent reviews per slide.

†$P<.001$ for all kappas unless otherwise noted.

‡Note that none of the nine slides had final diagnoses of benign with atypia.

by the United Kingdom National Breast Screening Programme in 1994 and involved up to 251 pathologists reviewing multiple sets of slides over 3 years *(5)*. As in our study, a high level of diagnostic consistency was achieved for most major categories of breast disease except when distinguishing benign with atypia and noninvasive, malignant categories. However, the slide sets did not represent the routine breast pathology caseload and slide quality was not formally assessed. The study of Bianchi et al. *(6)* showed good overall diagnostic agreement among 12 community-based Italian pathologists with comparable diagnostic discrepancies between benign with atypia and noninvasive malignant. However, although the study did control for the technical quality of the histologic sections, the cases selected for review were known to present diagnostic problems rather than randomly selected cases. In 1985, similar conclusions regarding diagnostic consistency were drawn from the study by members of the Medical Research Council Breast Tumor Pathology Panel in the U.K. who evaluated 40 consecutive cases submitted from health districts throughout the U.K. *(7)*.

Until more specific differentiating morphometric criteria or a biologic marker are determined, borderline proliferative breast lesions (representing 10% of our pathology database) will continue to be interpreted variably by community-based and expert pathologists alike. The natural history of low-grade noninvasive lesions as compared with the benign but atypical lesions is poorly understood. If the outcome of future clinical trials is to recommend comparable treatments for these borderline lesions, then the necessity to distinguish reproducibly between them may be alleviated.

Large cooperative clinical trials, such as the National Surgical Adjuvant Breast and Bowel Project, have tried to minimize inconsistencies of their pathologic findings by requiring that a central laboratory review all pathologic materials submitted by institutional pathologists *(9)*. Unless the clinical trials are specifically focusing on known areas of diagnostic variation, this procedure may not be necessary if the results of our current New Hampshire study apply broadly to pathologists elsewhere.

Two studies *(10,11)* have stated that optimal tissue fixation and processing are major factors in improving interobserver agreement in the histologic grading of breast carcinomas. In our study, reduced slide quality did not appear to affect diagnostic accuracy; indeed, for slides classified as of unsatisfactory interpretive quality or rated by greater than or equal to 25% as only satisfactory, the kappa coefficient improved from 0.64 to 0.69.

Three potential limitations of this study merit consideration. First, while the participation rate was good (80% of eligible pathologists submitting information to the pathology database and completing some aspects of the study), only 59% completed the slide review portion of the study. Willingness to take part in such a slide review may be considered a potential bias in participant selection and result in increased accuracy and agreement as compared with the community as a whole. Second, just one representative slide per case was requested for review, increasing the potential for sampling variability. In routine daily practice, pathologists would evaluate more than one slide from excisional and mastectomy specimens. Third, the uniform reporting form may have influenced final interpretations, since its format discouraged wordy comments.

In summary, breast pathology diagnoses among community pathologists in New Hampshire are highly reliable overall, particularly for the benign versus malignant categories, and for core biopsy specimens and special type invasive tumors. Tissue processing and slide quality do not measurably affect diagnostic agreement. Rereading breast pathology cases in categories critically important for determining treatment plans (benign with atypia and noninvasive malignant categories) only occurs in about 74% and 79% of the cases, respectively. A consistent slide review policy for breast pathology could lessen the likelihood of misclassification error. Clinically relevant diagnostic disagreements still occur, however, among noninvasive malignant diagnoses. The willingness of so many New Hampshire pathologists to participate in this project attests to their continued commitment to address these diagnostic variations and minimize clinically significant disagreements.

References

(1) Ernster V, Barclay J, Kerlikowske K, Grady D, Henderson C. Incidence of and treatment for ductal carcinoma in situ of the breast. JAMA 1996;12:913–8.

(2) Choi WS, Parker BA, Pierce JP, Greenberg ER. Regional differences in the incidence and treatment of carcinoma in situ of the breast. Cancer Epidemiol Biomarkers Prev 1996;4: 317–20.

(3) Rosai J. Borderline epithelial lesions of breast. Am J Surg Pathol 1991;15:209–21.

(4) Schnitt SJ, Connolly JL, Tavassoli FA, Fechner RE, Kempson R, Gelman R, et al. Interobserver reproducibility in the diagnosis of ductal proliferative breast lesions using standardized criteria. Am J Surg Pathol 1992;16:1133–43.

(5) Sloane JP, Ellman R, Anderson TJ, Brown CL, Coyne J, Dallimore NS, et al. Consistency of histopathological reporting of breast lesions detected by screening: findings of the U.K. National External Quality Assessment (EQA) Scheme. U.K. National Coordinating Group for Breast Screening Pathology. Eur J Can 1994;30A:1414–9

(6) Bianchi S, Palli D, Galli M, Arisio B, Cappa A, Dal Forno S, et al. Reproducibility of histological diagnoses and diagnostic accuracy of nonpalpable breast lesions. Pathol Res Pract 1994; 1:69–76.

(7) Beck JS. Observer variability in reporting of breast lesions. J Clin Pathol 1985;38:1358–65.

(8) Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977;33:159–74.

(9) Fisher ER, Costantino J. Quality assurance of pathology in clinical trials. The National Surgical Adjuvant Breast and Bowel Project experience. Cancer 1994;74(9 Suppl):2638–41.

(10) Robbins P, Pinder S, de Klerk N, Dawkins H, Harvey J, Sterrett G, et al. Histological grading of breast carcinomas: a study of interobserver agreement. Hum Pathol 1995;26:873–9.

(11) Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 1991;19:304–10.

Notes

Supported by The Centers for Disease Control to the New Hampshire Division of Health and Human Services (411216); by the U.S. Department of Defense (DASD17-94-J-4109); and by Public Health Service grant CA23108 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

We thank Dr. David Donsker for encouraging this project and Julie Wade, Dr. Marguerite M. Stevens, and Sarah Meyers for editorial assistance. We also greatly appreciate all New Hampshire pathologists who took part in this Project.

Manuscript received June 12, 1997; revised October 15, 1997; accepted October 29, 1997.

Identification of Melanoma Antigens That Are Immunogenic in Humans and Expressed *In Vivo*

*Jason Applebaum, Sandra Reynolds, Jeff Knispel, Ruth Oratz, Richard Shapiro, Jean-Claude Bystryn**

Background: **In the development of an antimelanoma vaccine, a critical factor is the identification of antigens that induce a strong immune response in humans and that are expressed by melanoma cells *in vivo*. The aim of this study was to identify candidate antigens for such vaccine. *Methods:* Sixty-nine patients with surgically resected melanomas (American Joint Commission on Cancer [AJCC] stage III) were immunized with a polyvalent vaccine containing multiple melanoma antigens. Antimelanoma antibodies generated in the patients' sera were used as probes to identify the melanoma antigens that are immunogenic in humans and that are expressed on the tumor tissue *in vivo*. Such responses were determined by an immunoblotting assay that employed an antigen source prepared from membrane fractions of freshly excised melanoma tissue. *Results and Conclusions:* Vaccine treatment stimulated antibody responses in 35 (51%; 95% confidence interval [CI] = 39%–63%) of 69 sequentially enrolled patients. The antibodies were directed to one or more antigens with molecular masses of 45, 59, 68, 79, 89, 95, and/or 110 kd. The most immunogenic antigens were p110 and p68, which induced responses in 33% (95% CI = 22%–44%) and 25% (95% CI = 15%–35%) of patients, respectively. Both antigens were commonly expressed on different melanomas, but they were absent on autologous normal tissue and on an unrelated allogeneic tumor. All the above antigens are attractive candidates for vaccine construction. [J Natl Cancer Inst 1998;90: 146–9]**

A major challenge in the design of effective vaccines for melanoma is the identification of candidate melanoma antigens for vaccine development *(1)*. The minimal essential requirements for such antigens are that they be immunogenic in humans and be expressed by melanoma cells *in vivo*. Two major approaches are currently being used to identify candidate antigens for vaccines; neither of these approaches is completely satisfactory. The first approach involves identifying antigens expressed on surgically resected melanoma tissue *in vivo* that are reactive with ''natural'' melanoma-specific T cells or melanoma-specific antibodies present in patients with this cancer *(2–5)*. Unfortunately, while this approach identifies molecules that are antigenic (i.e., able to react with an immune cell or antibody), it does not provide direct evidence of immunogenicity (i.e., the ability to stimulate an immune response in humans). The second approach is to directly identify antigens that are immunogenic in humans, as evidenced by their ability to stimulate an immune response in patients immunized to the antigen *(6–10)*. With this approach, however, the source of antigen for immune assays is usually the cells or antigen extract that was used to prepare the vaccine; therefore, it is difficult to exclude the possibility that the induced responses are directed against artifacts in the vaccine preparation. Neither approach identifies antigens that can stimulate tumor-protective immune response, which can be evaluated only by subsequent analysis of the effects of active immunization to the antigen on tumor progression.

This report describes the identification of multiple melanoma antigens that are both immunogenic in humans and expressed *in vivo*. The strategy used to achieve that goal was to employ vaccine-induced antibodies as probes to identify the immunogenic proteins in melanoma tissues. Extracts prepared from fresh, surgically resected melanoma tissue were used as a source of target antigens expressed *in vivo* and to avoid detection of antibodies to antigens that may be artifacts of the vaccine construction proteins. A polyvalent vaccine that contains a broad range of potential immunogens was used to detect and to compare the immunogenic potency of different antigens.

Materials and Methods

Melanoma Vaccine

A soluble, partially purified, polyvalent, melanoma antigen vaccine was prepared from the material shed into culture by four human melanoma cell lines (SF-SKMel28, SF-M14, SF-M20, and SF-HM54) that were adapted to long-term growth in serum-free medium, as previously described *(11,12)*. Briefly, these cells were incubated at 2×10^6/mL in serum-free RPMI-1640 medium for 3 hours at 37 °C. The spent culture medium from each cell line was collected, centrifuged at 1000*g* for 15 minutes at 4 °C to remove particulate material, and concentrated, and equal protein amounts of the concentrated shed material were collected from the four cell lines pooled. The non-ionic detergent Nonidet P-40 was added to a final concentration of 0.5% to the pooled shed material, which was then ultracentrifuged at 100 000*g* for 90 minutes at 4 °C to remove insoluble material, dialyzed against normal saline, and sterilized by filtration through a 0.22-μm filter. The protein concentration of the final product was adjusted to 0.2 mg/mL and dispensed into pyrogen-free glass vials. For use, the vaccine was usually admixed with alum as an adjuvant. The biochemical and antigenic properties of the vaccine have been published *(12)*.

Patients and Immunizations

Sixty-nine sequentially registered patients (43 men and 26 women between the ages of 18 and 75 years) with surgically resected melanoma (American Joint Commission on Cancer [AJCC] stage III) were enrolled in this study, which was conducted at the New York University Medical Center from 1992 to 1995. Other criteria for patient selection were as follows: no evidence of distant metastatic disease, no history of other cancers or other serious systemic disease, positive skin test response to recall antigens or ability to be sensitized to dinitrochlorobenzene, and no prior therapy for melanoma other than surgery or local radiotherapy. All patients signed informed consent. The patients were immunized with 20–40 μg of vaccine protein administered intradermally every 3 weeks four times and then monthly three times and at longer intervals thereafter. No other therapy for melanoma or immunosuppressive agents were given to the patients while they were being treated with the vaccine. Sera were collected from the patients before the first immunization and 1 week following six to eight immunizations. The sera were stored at −80 °C until used.

**Affiliations of authors:* J. Applebaum (Kaplan Cancer Center), S. Reynolds, J.-C. Bystryn (The Ronald O. Perelman Department of Dermatology), J. Knispel, R. Oratz (Department of Medicine), R. Shapiro (Department of Surgery), New York University School of Medicine, New York.

Correspondence to: Jean-Claude Bystryn, M.D., The Ronald O. Perelman Department of Dermatology, New York University Medical Center, 550 First Ave., New York, NY 10016.

See ''Notes'' following ''References.''

Preparation of Antigen Extracts for Immunoblotting

Two lots of melanoma antigen extracts were prepared; each was from equal volumes of surgically resected tumor tissue obtained from two separate patients. Lot 1 was prepared from hepatic metastases resected from one patient and from splenic metastases resected from a second patient; lot 2 was prepared from metastases resected from the chest wall of one patient and from the large intestine in a second patient. To prepare tumor tissue extracts, we separated the melanoma tissue from surrounding normal tissue and removed necrotic material. The tumor tissue was finely diced and homogenized, and nuclear and other cellular debris was removed by three cycles of centrifugation at 1000*g* for 10 minutes at 4 °C. The supernatants were ultracentrifuged at 105 000*g* for 1 hour at 4 °C, and the pellets were resuspended in Tris–EDTA buffer (pH 8.0) and ultracentrifuged again at 105 000*g* for 20 minutes at 4 °C. The resulting pellets were solubilized in 6 m*M* deoxycholic acid and stored at −80 °C.

A normal autologous tissue extract was prepared similarly from equal volumes of normal uninvolved liver and spleen obtained from the patient used to prepare melanoma antigen extract lot 1. A similar procedure was used to prepare antigen extracts from a control human parotid mixed tumor. Normal tissue from the patient from whom lot 2 was prepared was not available. Total protein concentration in all lots of antigens was measured by use of a Bio-Rad kit (Bio-Rad Laboratories, Paris, France) and normalized to 240 μg/gel.

Assay of Melanoma Antibodies

Antibodies to melanoma and the identity of antigens to which they were directed were determined by immunoblotting. Antigen extracts (240 μg of protein) were admixed with Laemmli's buffer *(13)* and 2-mercaptoethanol, boiled for 5 minutes, and centrifuged at 6400*g* for 2 minutes at 4 °C. The entire aliquot was run on sodium dodecyl sulfate–8% polyacrylamide gel electrophoresis *(13)*. Proteins were transferred onto the PVDF (Immobilon-P; Millipore Corp., Bedford, MA) membrane in 0.192 *M* glycine and 0.025 *M* Tris (pH 8.3) without methanol, blocked in 5% low-fat milk, and washed three times in 0.05% Tween 20 in phosphate-buffered saline (PBS). The membrane was then cut into equal strips (each strip containing 10 μg of protein) and incubated overnight in a 1:50 dilution of the patient's serum (unabsorbed). The strips were washed seven times with 0.05% Tween 20 in PBS, incubated for 4 hours in a 1:100 dilution of horseradish peroxidase-conjugated anti-human antibody [$F(ab')_2$ fragment; Sigma Chemical Co., St. Louis, MO] in 0.3% Tween 20 in PBS, washed seven times with 0.05% Tween in PBS, and incubated with a substrate containing 0.01% hydrogen peroxide and 0.5 mg/mL 4-chloro-1-naphthol. Vaccine-induced antibodies were evidenced by bands that were present in serum from the patient after vaccine treatment but absent or present in lesser density (≤50% decrease) in baseline serum obtained from the same patient before vaccine treatment. Approximate 95% confidence intervals (CIs) were generated by use of the formula

$$\left(p \pm 1.96\sqrt{\frac{p \times q}{n}}\right),$$

where p and q are the proportions with and without antibody responses, respectively, and n is the total number of patients, i.e., 69.

Results

The ability of vaccine treatment to stimulate antibodies to melanoma antigens expressed *in vivo* was investigated by a comparison of the pattern and level of melanoma antibodies in sera collected from 69 patients with surgically resected AJCC stage III malignant melanoma prior to vaccine treatment and 1 week after six to eight immunizations. The same melanoma antigen extract (lot 1), pooled from metastases resected from two patients, was used for all assays. The results are illustrated in Fig. 1 and summarized in Table 1. Vaccine treatment induced a new antibody response, or augmented a preexisting response, to one or more antigens expressed in surgically resected melanoma tissue in 35 (51% [95% CI = 39%–63%]) of 69 patients. The antibodies were directed to antigens of molecular masses of 45, 59, 68, 79, 89, 95, and/or 110 and 68 kd. Vaccine-induced immune responses were directed most commonly to the p110 antigen and to the p68 antigen (in 33% [95% CI = 22%–44%] and 25% [95% CI = 15%–35%] of patients, respectively) and least commonly to the p89 antigen and to the p45 antigen (in 10% [95% CI = 3%–17%] and 15% [95% CI = 7%–23%] of patients, respectively).

The specificity of the antigens defined by vaccine-induced antibodies was investigated by the measurement of their expression in extracts of surgically resected normal tissue and non-melanoma tumor, by use of a post-vaccine treatment serum with high levels of antibodies to the target antigens as a probe. The tissues tested included normal autologous tissues obtained from sites (liver in one patient and spleen in the other) adjacent to the metastases used to prepare melanoma antigen extract lot 1, another melanoma antigen extract (lot 2) prepared from metastases resected from two other patients, and an unrelated tumor (a parotid mixed tumor). All extracts were prepared identically and tested at identical protein concentrations. The results are illustrated in Fig. 2 and summarized in Table 2. None of the melanoma antigens targeted by these antibodies were detected in the autologous normal tissue. With the exception of antigens p68 and p89, none were detected in extracts of the parotid mixed tumor. The antigens were commonly expressed in melanoma, inasmuch as all were detected in a second lot of melanoma antigen extract (lot 2) prepared from two additional patients.

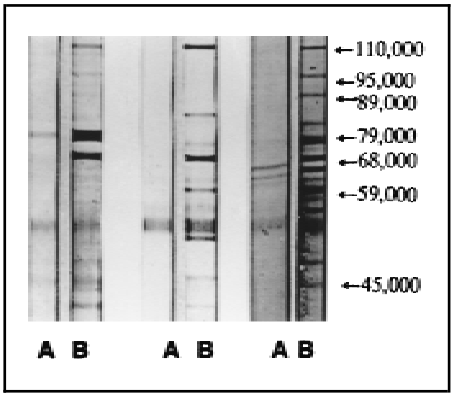

Fig. 1. Melanoma antigens defined by preimmunization and vaccine-induced antibodies. Sera were collected from three patients before vaccine treatment (**lanes A**) and 1 week after six to eight immunizations (**lanes B**). The sera were tested for antibodies to membrane extracts of fresh melanoma tissue by western immunoblot analysis. Before vaccine treatment, most patients had antibodies to one or more antigens. After vaccine treatment, the patients developed new or increased levels of antibodies to one or more antigens with molecular masses of 45, 59, 68, 79, 89, 95, and/or 110 kd.

Discussion

This study shows that immunization to a polyvalent melanoma vaccine prepared from antigens shed from melanoma cells cultured *in vitro* induces antibody responses to multiple melanoma antigens of molecular masses 45, 59, 68, 79, 89, 95, and/or 110 kd that are expressed *in vivo* in fresh melanoma tissue. This observation demonstrates that the vaccine contains multiple antigens that are immunogenic in humans and that the antimelanoma antibody responses that these antigens induce are not directed to artifactual antigens; moreover, it identifies these antigens as candidates for construction of melanoma vaccines.

There is presently considerable interest in the development of vaccines to treat, and possibly to prevent, some cancers. To be effective, such vaccines must be able to stimulate antitumor immune responses directed to antigens that are expressed *in vivo* by the tumor. This requires that the vaccine contains tumor antigens that are both immunogenic in humans and expressed *in vivo (1)*. In the

Table 1. Generation of vaccine-induced antibodies that react with antigens present in fresh melanoma tissue

Melanoma antigen, kd	Patients* with antibody response to melanoma vaccine immunization†		
	Vaccine-induced responses, No. (%)‡	Vaccine-enhanced responses, No. (%)§	Any antibody response to immunization,‖ No. (%; 95% confidence interval)
110	9 (13)	14 (20)	23 (33; 22–44)
95	10 (15)	5 (7)	15 (22; 12–32)
89	2 (3)	5 (7)	7 (10; 3–17)
79	9 (13)	2 (3)	11 (16; 7–25)
68	10 (15)	7 (10)	17 (25; 15–35)
59	9 (13)	6 (9)	15 (22; 12–32)
45	8 (12)	2 (3)	10 (15; 7–23)
Any antigen	23 (33)	24 (35)	35 (51; 39–63)

*Total number of patients = 69.

†Measured by immunoblot analysis, with the use of a membrane fraction of fresh melanoma tissue as the antigen source.

‡Antibody band present in post-treatment but not pretreatment serum in same patient.

§Density of antibody band greater in post-treatment serum than in pretreatment serum.

‖Approximate 95% confidence intervals were generated by use of the formula

$$\left(p \pm 1.96 \sqrt{\frac{p \times q}{n}}\right),$$

where p and q are proportions with and without antibody responses, respectively, and n is 69, i.e., the total number of patients.

case of malignant melanoma, the identity of such antigens remains to be fully defined.

Few melanoma antigens satisfy the criteria of being both immunogenic in humans and expressed *in vivo* by melanoma. These include the GM2 and GD2 gangliosides *(8,14)*. These gangliosides are weakly immunogenic, requiring conjugation to a carrier protein and administration with potent adjuvants to induce long-lasting antibody responses in patients *(15)*. Other melanoma antigens present in vaccines are immunogenic in humans, as determined by the induction of antibodies, but their expression *in vivo* is unknown. These include a number of antigens ranging in molecular masses from 34- to100-kd antibodies that were induced by immunization to a melanoma vaccinia viral oncolysate *(6);* a 31-kd glycoprotein antigen to which an antibody was induced by a vaccinia virus melanoma oncolysate *(9);* and cell surface antigens of 38–43, 75, 110, 150, and 210 kd induced by the polyvalent shed antigen vaccine used in the current study *(7)*. However, in all these cases, the antigen source used to detect vaccine-induced antibodies was derived from cultured cells, making it difficult to exclude the possibility that the antibodies were induced and directed to artifacts of tissue culture rather than being expressed *in vivo.* Other melanoma antigens are known to be expressed *in vivo* and to react with human T cells obtained from patients with melanoma, but their immunogenicity *in vivo* is unknown. These include the MAGE-1, MAGE-3, and MART-1 peptides *(3,4),* gp100 *(16),* and tyrosinase *(17),* all of which are recognized by human leukocyte antigen (HLA)-restricted cytotoxic lymphocytes obtained from patients with melanoma. However, although these studies show that these molecules are antigenic *in vitro,* they do not establish with certainty whether they are immunogenic *in vivo* because the lymphocytes could have been sensitized by different or cross-reacting antigens.

In this study, our strategy to identify antigens that are both immunogenic in humans and expressed *in vivo* has been to use as probes antibodies induced by vaccine immunization. The detection of several antigens that satisfy this definition was made feasible by immunization of patients to a polyvalent melanoma vaccine that contains a broad range of potential immunogens. A large number of patients was studied (n = 69) to permit the evaluation of relative immunogenicity of the antigens and the identification of

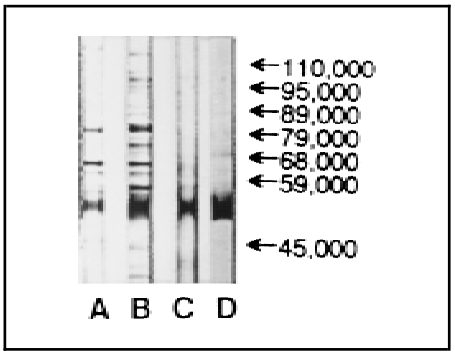

Fig. 2. Specificity analysis of vaccine-induced antibodies against melanoma antigens expressed *in vivo.* Serum of one representative patient with vaccine-induced antibodies was tested for reactivity against two different lots of resected melanoma tissue prepared from different patients (**lanes A and B**), pooled autologous normal tissue obtained from a site adjacent to that of the melanoma tissue used in lane B (**lane C**), and an unrelated parotid mixed tumor (**lane D**). Membrane extracts of all tissues were prepared in a similar manner and were tested at similar protein concentrations.

Table 2. Tissue distribution of melanoma antigens defined by antibodies induced by vaccine immunization

Melanoma antigen, kd	Antigen expression*			
	Melanoma antigen lot 1†	Melanoma antigen lot 2‡	Normal autologous tissue§	Unrelated tumor‖
95	+	+++	−	−
89	++	+++	−	+
79	++	++	−	−
68	+++	+++	−	+
59	++	++	−	Not tested
51	++	+++	−	−
45	+++	+++	−	−

*The number of + signs are indicative of relative band density on immunoblots. + = the lowest; +++ = the highest; − = absence of the band(s).

†Prepared from metastatic nodules obtained from the liver and spleen of two different patients.

‡Prepared from metastatic nodules obtained from the chest wall and bowel of two different patients.

§Normal tissue obtained from autologous liver and spleen from patients utilized for the preparation of melanoma pool #1.

‖Parotid mixed tumor.

weakly immunogenic antigens that induce responses in only a small proportion of patients. The latter antigens are still potentially valuable for vaccine development because the immunogenicity of even weakly immunogenic entities can be markedly increased by appropriate immunizing strategies *(15)*.

Our results indicate that a large number of antigens expressed by melanoma cells *in vivo* can be immunogenic in humans. These include proteins with molecular masses of 45, 59, 68, 79, 89, 95, and 110 kd. Vaccine-induced antibody responses to one or more of these antigens were detected in approximately half of 69 sequentially registered patients with surgically resected stage III melanoma. The immunodominant antigens (p110 and p68) were those of molecular masses of 110 kd and 68 kd and stimulated antibody responses in 33% and 25% of patients, respectively. All of these antigens appear to be melanoma associated, inasmuch as none could be detected in normal tissue. They appear to be common melanoma antigens because all could be detected in different melanomas. The 89-kd and 68-kd antigens were weakly expressed on an unrelated cancer. The relation of these antigens to previously described melanoma antigens remains to be defined. However, all but p45 are unrelated to HLA antigens on the basis of their molecular size. If these antigens are shown to differ from currently known melanoma antigens, the sera used to identify them could be used for cloning them.

In summary, we have demonstrated that a polyvalent melanoma vaccine prepared from antigens shed from cultured melanoma cell lines can stimulate immune responses to multiple melanoma antigens expressed *in vivo*. These antigens are attractive candidates for the development of melanoma vaccines because they possess the dual property of being immunogenic in humans and selectively expressed by melanoma cells *in vivo*.

References

(1) Bystryn JC, Shapiro RL, Oratz R. Cancer vaccines: clinical applications: partially purified tumor antigen vaccines. In: DeVita VT Jr, Hellman S, Rosenberg SA, editors. Biologic therapy of cancer. 2nd ed. Philadelphia: Lippincott, 1995:668–79.

(2) Lloyd K. Tumor antigens. Ann N Y Acad Sci 1993;690:50–9.

(3) Gaugler B, Van den Eynde B, van der Bruggen P, Romero P, Gaforio JJ, De Plaen E, et al. Human gene MAGE-3 codes for an antigen recognized on a melanoma by autologous cytolytic T lymphocytes. J Exp Med 1994;179: 921–30.

(4) Kawakami Y, Eliyahu S, Sakaguchi K, Robbins PF, Rivoltini L, Yannelli JR, et al. Identification of the immunodominant peptides of the MART-1 human melanoma antigen recognized by the majority of HLA-A2-restricted tumor infiltrating lymphocytes. J Exp Med 1994; 180:347–52.

(5) Oka T, Kikumoto Y, Itakura K, Morton DL, Irie RF. Human monoclonal antibody identified an immunoreactive tetrapeptide sequence (Lys–Tyr–Gln–Ile) in Mr 43,000 protein of human melanoma. Cancer Res 1994;54:3511–5.

(6) Hersey P, Werkman H, Edwards AE. Western blot analysis of serological responses following immunization with vaccinia viral lysates of melanoma cells. Int J Cancer 1990;46:612–7.

(7) Bystryn JC, Henn M, Li J, Shroba S. Identification of immunogenic human melanoma antigens in a polyvalent melanoma vaccine. Cancer Res 1992;52:5948–53.

(8) Livingston PO. Approaches to augmenting the IgG antibody response to melanoma ganglioside vaccines. Ann N Y Acad Sci 1993;690: 204–13.

(9) Berthier-Vergnes O, Portoukalian J, Leftheriotis E, Dore JF. Induction of IgG antibodies directed to a Mr 31,000 melanoma antigen in patients immunized with vaccinia virus melanoma oncolysates. Cancer Res 1994;54: 2433–9.

(10) Tai T, Cahan LD, Tsuchida T, Saxton RE, Irie RF, Morton DL. Immunogenicity of melanoma-associated gangliosides in cancer patients. Int J Cancer 1985;35:607–12.

(11) Bystryn JC, Oratz R, Roses D, Harris M, Henn M, Lew R. Relation between immune response to melanoma vaccine immunization and clinical outcome in stage II malignant melanoma. Cancer 1992;69:1157–64.

(12) Bystryn JC, Jacobsen S, Harris M, Roses D, Speyer J, Levin M. Preparation and characterization of a polyvalent human melanoma antigen vaccine. J Biol Response Mod 1986;5: 221–4.

(13) Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;2127:680–5.

(14) Livingston PO, Kaelin K, Pinsky CM, Oettgen HF, Old LJ. The serological response of patients with stage II melanoma to allogeneic melanoma cell vaccines. Cancer 1985;56: 2194–200.

(15) Helling F, Adluri S, Calves M, Koganty RR, Oettgen HF, Livington PO. Increased immunogenicity of GM2 conjugated with KLH and used with adjuvant in patients with melanoma. Proc Am Assoc Cancer Res 1993;34:491.

(16) Bakker AB, Schreurs MW, de Boer AJ, Kawakami Y, Rosenberg SA, Adema GJ, et al. Melanocyte lineage-specific antigen gp100 is recognized by melanoma-derived tumor-infiltrating lymphocytes. J Exp Med 1994;179: 1005–9.

(17) Wolfel T, Van Pel A, Brichard V, Schneider J, Selliger B, Meyer zum Buschenfelde KH, et al. Two tyrosinase nonapeptides recognized on HLA-A2 melanomas by autologous cytolytic T lymphocytes. Eur J Immunol 1994;24:759–64.

Notes

Supported in part by Public Health Service grants P30CA16087, 1RO1CA58484, and 1RO1CA60783 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services; by grant FD-R-000632 from the U.S. Food and Drug Administration; and by grants from the Rose M. Badgeley Residuary Trust and the Catherine and Henry Gaisman Foundation.

Manuscript received March 17, 1997; revised September 19, 1997; accepted October 30, 1997.

Body Mass Index and Risk of Adenocarcinomas of the Esophagus and Gastric Cardia

*Wong-Ho Chow, William J. Blot, Thomas L. Vaughan, Harvey A. Risch, Marilie D. Gammon, Janet L. Stanford, Robert Dubrow, Janet B. Schoenberg, Susan T. Mayne, Diana C. Farrow, Habibul Ahsan, A. Brian West, Heidi Rotterdam, Shelley Niwa, Joseph F. Fraumeni, Jr.**

Background: **Incidence rates have risen rapidly for esophageal adenocarcinoma and moderately for gastric cardia adenocarcinoma, while rates have remained stable for esophageal squamous cell carcinoma and have declined steadily for noncardia gastric adenocarcinoma. We examined anthropometric risk factors in a population-based case–control study of esophageal and gastric cancers in Connecticut, New Jersey, and western Washington.** ***Methods:*** **Healthy control subjects (n = 695) and case patients with esophageal squamous cell carcinoma or noncardia gastric adenocarcinoma (n = 589) were frequency-matched to case patients with adenocarcinomas of esophagus or gastric cardia (n = 554) by 5-year age groups, sex, and race (New Jersey only). Classification of cases by tumor site of origin and histology was determined by review of pathology materials and hospital records. Data were collected using in-person structured interviews. Associations with obesity, measured by body mass index (BMI), were estimated by odds ratios (ORs). All ORs were adjusted for geographic location, age, sex, race, cigarette smoking, and proxy response status.** ***Results:*** **The ORs for esophageal adenocarcinoma rose with increasing adult BMI. The magnitude of association with BMI was greater among the younger age groups and among nonsmokers. The ORs for gastric cardia adenocarcinoma rose moderately with increasing BMI. Adult BMI was not associated with risk of esophageal squamous cell carcinoma or noncardia gastric adenocarcinoma.** ***Conclusions:*** **Increasing prevalence of obesity in the United States population may have contributed to the upward trends in esophageal and gastric cardia adenocarcinomas. [J Natl Cancer Inst 1998;90:150–5]**

The incidence of esophageal adenocarcinoma has been rapidly rising over the past two decades in the United States and western Europe *(1–5).* To a lesser extent, increases in the incidence of gastric cardia adenocarcinoma have also been reported *(2,6–8).* In contrast, incidence rates for squamous cell carcinoma of the esophagus have remained stable or decreased slightly, while rates for noncardia gastric adenocarcinoma have declined steadily. To identify reasons for the upward trend in esophageal and gastric cardia adenocarcinomas, we conducted a population-based case–control study of these tumors in three areas of the United States. In the initial report from this study, cigarette smoking was found to be a risk factor *(9).* The present analysis evaluates the possible role of excess weight, which has been suggested as a risk factor in previous studies *(10–12).*

Methods

The methods for this study are described in detail elsewhere *(9).* Briefly, residents newly diagnosed with invasive esophageal or gastric cancers at ages 30–79 years in Connecticut (from February 1, 1993, to January 31, 1995), New Jersey (from April 1, 1993, to November 30, 1994), and western Washington (from March 1, 1993, to February 28, 1995) were identified through rapid reporting systems. Population-based control subjects were selected by random digit dialing *(13)* for those under 65 years of age and from the Health Care Financing Administration files for those 65 years of age or older *(14).* Healthy control subjects and case patients with esophageal squamous cell carcinoma or noncardia gastric adenocarcinoma (comparison cases) were frequency matched to target case patients with adenocarcinomas of the esophagus or gastric cardia, including the gastroesophageal junction, in each geographic area by 5-year age group and sex and in New Jersey by race (white or non-white). Classification of cases by site of origin and histology was determined by a panel of pathologists through standardized review of pathology materials and reports from surgery, endoscopy, and radiology.

After obtaining written informed consent from each subject or next of kin of a deceased subject, an in-person, structured interview was conducted to elicit information on demographic background, tobacco and alcohol use, medication and medical histories, diet, occupation, and height and weight history up to 1 year prior to diagnosis for case patients and date of interview for control subjects. Weight history included usual adult weight (i.e., the most common weight during adulthood), highest adult weight, and usual weights during ages 20–29, 40–49 and 60–69. Interviews were obtained for 554 (81%) of 687 eligible target case patients, 589 (74%) of 795 eligible comparison case patients, and 695 (74%) of 943 eligible control subjects. Of these, information was provided by next of kin for 164 (30%) of the target case patients, 192 (33%) of the comparison case patients, and 25 (4%) of the control subjects.

Adiposity was estimated by body mass index (BMI), computed as weight in kilograms divided by height in meters squared (kg/m^2). Height, weight, and BMI variables were grouped into quartiles for analysis based on sex-specific distributions among the control subjects. Anthropometric variables more finely grouped in deciles also were examined for linearity of associations. Relative risks according to anthropometric status were estimated by odds ratios (ORs) and 95% confidence intervals (CIs), using logistic regression models *(15).* The CIs were not adjusted for multiple comparisons. Dose–response relationships were evaluated by tests of linear trend based on continuous variables. Effect modification was assessed by examination of stratum-specific results. The significance of the interaction was tested by adding a cross-product term to the model. All ORs were adjusted for geographic location, age, sex, race (white, non-white), cigarette smoking (nonsmoker, former smoker at 1 or more years prior to interview, and current smoker) and respondent status (self and next of kin). Separate analyses using more detailed cigarette smoking indicators, including pack-years of smoking, combination of pack-years and smoking status, and years since smoking cessation for past smokers, did not alter the associations. Additional adjustment for other potential confounding factors, including family income, education, dietary intake (calories, fat, or fiber), level of

**Affiliations of authors:* W. H. Chow, J. F. Fraumeni, Jr., Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD; W. J. Blot, International Epidemiology Institute, Rockville, MD; T. L. Vaughan, J. L. Stanford, D. C. Farrow, Program in Epidemiology, Fred Hutchinson Cancer Research Center, and Department of Epidemiology, University of Washington School of Public Health, Seattle; H. A. Risch, R. Dubrow, S. T. Mayne, Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT; M. D. Gammon, H. Ahsan, Division of Epidemiology, Columbia University School of Public Health, New York, NY; J. B. Schoenberg, Applied Cancer Epidemiology Program, New Jersey Department of Health and Senior Services, Trenton; B. West, Department of Pathology, Yale University School of Medicine; H. Rotterdam, Department of Pathology, College of Physicians & Surgeons of Columbia University, New York, NY; S. Niwa, Westat, Inc., Rockville, MD.

Correspondence to: Wong-Ho Chow, Ph.D., National Institutes of Health, Executive Plaza North, Bethesda, MD 20892.

See ''Notes'' following ''References.''

physical activity, alcohol use, history of reflux disease, usual occupational categories, or family history of cancer, did not materially alter the risk estimates.

Results

Initial analyses were conducted to examine the consistency of results based on all study subjects and after excluding next-of-kin interviews. Since the associations with height, usual weight, and usual BMI were similar with and without the next-of-kin interviews, results are presented for the entire study population. The findings also were unchanged when nonwhites were excluded from the analyses. In addition, the patterns of risks were similar between men and women, hence the results are presented for both sexes and all races combined. Presented in Table 1 are ORs associated with usual BMI for men and women separately as well as ORs after excluding next-of-kin interviews or non-whites.

As shown in Table 2, height tended to be inversely related to risk for all tumor types except esophageal squamous cell carcinoma, although the trend was statistically significant only for esophageal adenocarcinoma. High usual adult weight was associated with excess risks of adenocarcinomas of the esophagus and gastric cardia, reduced risk of esophageal squamous cell carcinoma, and no association with noncardia gastric adenocarcinoma. For usual BMI, the ORs for esophageal adenocarcinoma rose steadily. When compared with the first quartile, the OR increased from 1.3 (95% CI = 0.8–2.2) for the second quartile to 2.0 (95% CI = 1.3–3.3) and 2.9 (95% CI = 1.8–4.7) in the third and fourth quartiles, respectively (P for trend <.0001). Furthermore, compared with subjects in the lowest 10% of usual BMI (<21.70 for men and <20.18 for women), risk increased steadily to reach fivefold (OR = 5.4, 95% CI = 2.4–12.0) among those in the highest decile (≥29.54 for men and ≥31.25 for women). To a lesser extent, ORs for gastric cardia adenocarcinoma rose with usual BMI to 1.6 (95% CI = 1.1–2.6) in the highest quartile. Among those in the highest decile of usual BMI, the risk of gastric cardia adenocarcinoma increased twofold (OR = 2.1, 95% CI = 1.1–4.1) relative to those in the lowest decile. In contrast, usual BMI was not significantly related to risk of esophageal squamous cell carcinoma or noncardia gastric adenocarcinoma.

Risk of esophageal adenocarcinoma was not related to weight gain during adulthood, except when the gain was large (≥46 lbs or ≥21% since age 20 years) (Table 2). Stratification by usual BMI showed excess risks associated with weight gain greater than or equal to 46 lbs (OR = 1.7; 95% CI = 0.6–4.9) and weight gain of greater than or equal to 21% (OR = 1.5; 95% CI = 0.7–3.2) only among those in the highest quartile of usual BMI. Weight changes were not consistently associated with cardia or noncardia gastric adenocarcinoma, but risk of esophageal squamous cell carcinoma tended to increase with weight loss and decrease with weight gain since ages 20–29 years. For each tumor type, the risk pattern associated with BMI at ages 20–29, 40–49, and 60–69 years, or BMI based on the maximum adult weight, was similar to that of usual BMI (data not shown). The results were not altered substantially after excluding next-of-kin respondents, with a twofold excess risk (OR = 2.2; 95% CI = 1.2–4.0) for those who gained greater than or equal to 46 lbs and a 40% excess (OR = 1.4; 95% CI = 0.9–2.2) for those who gained greater than or equal to 21% of the weight during ages 20–29 years.

The joint effects of height and usual adult weight were further assessed for esophageal and gastric cardia adenocarcinomas (Table 3). Risk of esophageal adenocarcinoma generally rose with increasing weight at each height level, and declined with increasing height at each weight level. Similar but less consistent patterns were found for gastric cardia adenocarcinoma. The patterns of risk by

Table 1. Odds ratios (ORs) and 95% confidence intervals (CIs) associated with usual body mass index (BMI) by sex and respondent characteristics*

Stratum	Usual BMI	No. of control subjects	Esophageal adenocarinoma		Gastric cardia adenomcarcinoma		Esophogeal squamous cell carcinoma		Noncardia gastric adenocarcinoma	
			No.	OR (95% CI)†	No.	OR (95% CI)†	No.	OR (95% CI)†	No.	OR (95% CI)†
Men‡	I—low	138	36	1.0 (referent)	45	1.0 (referent)	64	1.0 (referent)	66	1.0 (referent)
	II	138	55	1.5 (0.8–2.5)	44	0.9 (0.6–1.6)	37	0.4 (0.2–0.8)	54	1.0 (0.6–1.7)
	III	141	72	2.0 (1.2–3.5)	59	1.3 (0.8–2.2)	39	0.7 (0.4–1.3)	65	1.4 (0.9–2.3)
	IV—high	138	81	3.0 (1.7–5.0)	75	1.8 (1.1–2.9)	35	0.7 (0.4–1.3)	66	1.4 (0.9–2.3)
Women‡	I—low	34	9	1.0 (referent)	9	1.0 (referent)	15	1.0 (referent)	39	1.0 (referent)
	II	35	8	0.8 (0.2–3.4)	7	0.9 (0.2–3.2)	13	0.6 (0.2–1.9)	23	0.7 (0.3–1.6)
	III	35	13	2.1 (0.6–7.4)	11	2.2 (0.7–7.1)	14	0.8 (0.3–2.3)	26	0.8 (0.3–1.8)
	IV—high	35	18	2.6 (0.8–8.5)	11	1.3 (0.4–4.2)	3	0.2 (0.0–0.8)	26	0.7 (0.3–1.5)
Excluded‡ next of kin	I—low	166	25	1.0 (referent)	40	1.0 (referent)	57	1.0 (referent)	57	1.0 (referent)
	II	164	38	1.4 (0.8–2.4)	38	0.9 (0.6–1.6)	25	0.5 (0.3–0.8)	58	1.1 (0.7–1.7)
	III	172	59	2.1 (1.2–3.5)	54	1.3 (0.8–2.1)	38	0.7 (0.4–1.2)	71	1.3 (0.9–2.0)
	IV—high	168	76	3.1 (1.8–5.2)	60	1.6 (1.0–2.5)	24	0.5 (0.3–0.9)	67	1.3 (0.8–2.0)
Excluded‡ non-whites	I—low	156	45	1.0 (referent)	50	1.0 (referent)	60	1.0 (referent)	84	1.0 (referent)
	II	162	62	1.3 (0.8–2.1)	51	0.9 (0.6–1.5)	36	0.4 (0.3–0.8)	64	0.9 (0.6–1.4)
	III	169	84	1.9 (1.2–3.2)	67	1.3 (0.8–2.1)	41	0.7 (0.4–1.2)	79	1.2 (0.8–1.8)
	IV—high	158	97	2.8 (1.7–4.5)	84	1.7 (1.1–2.6)	31	0.6 (0.3–1.1)	77	1.3 (0.8–1.0)

*Cut points for usual BMI: first quartile (males: <23.12; females: <21.95); second quartile (males: 23.12–25.08; females: 21.95–24.12); third quartile (males: 25.09–27.31; females: 24.13–27.43); and fourth quartile (males: ≥27.32; females: ≥27.44).

†Adjusted for geographic location, age, cigarette smoking, and sex, race, and respondent status (when appropriate).

‡One control subject, one esophageal adenocarcinoma patient, one esophageal squamous cell carcinoma patient, and three patients with noncardia adenocarcinoma were excluded because of missing values.

Table 2. Odds ratios (ORs) and 95% confidence intervals (CIs) for esophageal adenocarcinoma, gastric cardia adenocarcinoma, esophageal squamous cell carcinoma, and noncardia gastric adenocarcinoma in relation to anthropometric variables

Anthropometric variables	No. of controls	Esophageal adenocarinoma		Gastric cardia adenomcarcinoma		Esophogeal squamous cell carcinoma		Noncardia gastric adenocarcinoma	
		N*	OR (95% CI)†	N*	OR (95% CI)†	N*	OR (95% CI)†	N*	OR (95% CI)†
Adult height‡ (quartiles in inches)									
I—low (males: <68; females: <63)	149	73	1.0 (referent)	60	1.0 (referent)	51	1.0 (referent)	122	1.0 (referent)
II (males: 68–69; females: 63)	155	69	0.6 (0.4–1.0)	53	0.7 (0.4–1.2)	41	0.6 (0.4–1.2)	62	0.5 (0.3–0.7)
III (males: 70–71; females: 64–65)	174	76	0.6 (0.4–0.9)	66	0.8 (0.5–1.3)	68	1.4 (0.8–2.4)	95	0.7 (0.5–1.1)
IV—high (males: ⩾72; females: ⩾66)	216	74	0.4 (0.2–0.6)	82	0.6 (0.4–1.1)	61	1.0 (0.6–1.8)	87	0.6 (0.4–0.9)
P for trend§			.0001		.1450		.3139		.0567
Usual adult weight‖ (quartiles in lbs)									
I—low (males: <160; females: <128)	167	49	1.0 (referent)	51	1.0 (referent)	76	1.0 (referent)	123	1.0 (referent)
II (males: 160–176; females: 128–139)	189	86	2.1 (1.3–3.4)	63	1.2 (0.7–1.9)	70	0.9 (0.5–1.4)	94	0.9 (0.6–1.3)
III (males: 177–189; females: 140–159)	150	45	1.6 (0.9–2.8)	44	1.2 (0.7–2.0)	32	0.6 (0.3–1.1)	59	0.7 (0.5–1.2)
IV—high (males: ⩾190; females: ⩾160)	188	112	4.0 (2.4–6.7)	103	2.1 (1.3–3.5)	42	0.6 (0.3–1.1)	89	0.9 (0.6–1.4)
P for trend§			<.0001		.0016		.0462		.6607
Usual BMI¶ (quartiles)									
I—low (males: <23.12; females: <21.95)	172	45	1.0 (referent)	54	1.0 (referent)	79	1.0 (referent)	105	1.0 (referent)
II (males: 23.12–25.08; females: 21.95–24.12)	173	63	1.3 (0.8–2.2)	51	0.9 (0.6–1.5)	50	0.5 (0.3–0.9)	77	0.9 (0.6–1.4)
III (males: 25.09–27.31; females: 24.13–27.43)	176	85	2.0 (1.3–3.3)	70	1.4 (0.9–2.1)	53	0.8 (0.5–1.3)	91	1.2 (0.8–1.8)
IV—high (males: ⩾27.32; females: ⩾27.44)	173	99	2.9 (1.8–4.7)	86	1.6 (1.1–2.6)	38	0.6 (0.3–1.0)	92	1.2 (0.8–1.8)
P for trend§			<.0001		.0080		.1065		.2141
Weight change (age 20–29 y to usual adult)									
Loss/gain 0–5 lbs	192	67	1.0 (referent)	81	1.0 (referent)	75	1.0 (referent)	90	1.0 (referent)
Loss ⩾6 lbs	31	8	1.0 (0.4–2.3)	15	1.0 (0.5–2.0)	19	1.8 (0.9–3.6)	18	2.0 (1.0–3.9)
Gain 6–25 lbs	291	116	1.1 (0.7–1.6)	85	0.6 (0.4–0.9)	82	0.9 (0.6–1.4)	158	1.3 (0.9–1.9)
Gain 26–45 lbs	125	43	1.0 (0.6–1.7)	42	0.8 (0.5–1.2)	22	0.5 (0.3–1.0)	60	1.3 (0.8–2.1)
Gain ⩾46 lbs	54	31	2.1 (1.2–3.8)	30	1.3 (0.7–2.2)	7	0.3 (0.1–0.8)	27	1.4 (0.8–2.5)
Percent weight change (age 20–29 y to usual adult)									
−5 to +5%	245	83	1.0 (referent)	95	1.0 (referent)	92	1.0 (referent)	115	1.0 (referent)
Loss ⩾6%	22	5	0.8 (0.3–2.3)	12	1.3 (0.6–2.8)	13	1.6 (0.7–3.5)	10	1.4 (0.6–3.2)
Gain 6–20%	290	121	1.1 (0.8–1.6)	94	0.8 (0.5–1.1)	76	0.7 (0.5–1.1)	153	1.2 (0.8–1.6)
Gain ⩾21%	136	56	1.4 (0.9–2.1)	52	1.0 (0.6–1.5)	24	0.4 (0.2–0.7)	75	1.2 (0.8–1.9)

*The number of subjects do not add up to the total number interviewed because of missing values in some categories.

†Adjusted for geographic location, age, sex, race, cigarette smoking, and respondent status.

‡Also adjusted for usual adult weight.

§Based on continuous variables.

‖Also adjusted for adult height.

¶Body mass index based on usual adult weight, calculated as weight in kg/height in m^2.

Table 3. Odds ratios (ORs) and 95% confidence intervals (CIs) for esophageal and gastric cardia adenocarcinomas in relation to weight by height

Height (inches) quartiles	Weight (lbs) quartiles							
	I—low (males: <160; females: <128)		II (males: 160–176; females: 128–139)		III (males: 177–189; females: 140–159)		IV—high (males: >189; females: >159)	
	Case patients/ control subjects*	OR (95% CI)†	Case patients/ control subjects	OR (95% CI)†	Case patients/ control subjects	OR (95% CI)†	Case patients/ control subjects	OR (95% CI)†
Esophageal adenocarcinoma								
I—low (males: <68; females: <63)	29/74	1.0 (referent)	27/42	2.5 (1.2–5.3)	8/17	2.0 (0.7–5.8)	9/16	2.3 (0.8–6.6)
II (males: 68–69; females: 63)	10/47	0.7 (0.3–1.8)	26/48	1.4 (0.7–3.0)	11/32	1.0 (0.4–2.6)	22/28	2.5 (1.1–5.6)
III (males: 70–71; females: 64–65)	8/33	0.5 (0.2–1.6)	16/56	0.8 (0.3–1.7)	16/40	1.2 (0.5–2.8)	36/45	2.9 (1.4–5.9)
IV—high (males: ⩾72; females: ⩾66)	2/13	0.5 (0.1–3.1)	17/43	1.0 (0.5–2.3)	10/61	0.4 (0.1–1.0)	45/99	1.5 (0.8–2.9)
Gastric cardia adenocarcinoma								
I—low (males: <68; females: <63)	24/74	1.0 (referent)	18/42	1.7 (0.8–3.7)	8/17	2.0 (0.7–5.8)	10/16	1.8 (0.6–5.2)
II (males: 68–69; females: 63)	15/47	1.0 (0.4–2.2)	13/48	0.8 (0.4–1.9)	6/32	0.6 (0.2–1.8)	19/28	2.2 (1.0–5.0)
III (males: 70–71; females: 64–65)	10/33	1.1 (0.4–2.7)	16/56	0.9 (0.4–2.0)	15/40	1.3 (0.6–2.9)	25/45	2.0 (0.9–4.3)
IV—high (males: ⩾72; females: ⩾66)	2/13	0.8 (0.2–3.8)	16/43	1.0 (0.5–2.4)	15/61	0.8 (0.3–1.7)	49/99	1.6 (0.8–3.0)

*One control and one esophageal adenocarcinoma case were excluded because of missing values.

†Adjusted for geographic location, age, sex, race, cigarette smoking, and respondent status.

height and usual adult weight were unremarkable for esophageal squamous cell carcinoma and non-cardia gastric adenocarcinoma (data not shown).

The positive association between risk of esophageal adenocarcinoma and usual BMI was significantly ($P = .03$) modified by age (at the time of diagnosis for case patients and at the time of interview for control subjects), with the greatest increase in risk seen among the youngest group (ages <50 years) and the smallest increase among the oldest group (ages 70–79 years) (Table 4). The ORs for the highest quartile relative to the lowest quartile of usual BMI in age groups less than 50, 50–59, 60–69 and 70–79 years were 33.6 (95% CI = 2.1–552), 4.5 (95% CI = 1.4–14.1), 2.3 (95% CI = 1.0–5.4), and 1.7 (95% CI = 0.8–3.8), respectively. Effect modification by age was not apparent for gastric cardia adenocarcinoma (data not shown), although no relation to usual BMI was found among those aged 70 years and older, a pattern consistent with that for esophageal adenocarcinoma. Associations between usual BMI and risk of esophageal squamous cell carcinoma or noncardia gastric adenocarcinoma were not modified by age (data not shown).

Cigarette smoking, a risk factor for each of the cancers in our study, was a significant ($P = .03$) effect modifier of the risk of esophageal adenocarcinoma associated with usual BMI (Table 4). The largest BMI-related increase in risk was found among nonsmokers, followed by current smokers and then former smokers. The ORs for the highest versus the lowest quartile of usual BMI were 8.7 (95% CI = 2.4–31.1) among nonsmokers, 2.1 (95% CI = 1.1–4.2) among former smokers, and 2.9 (95% CI = 1.1–7.6) among current smokers. The effect of usual BMI on risk of gastric cardia adenocarcinoma was not significantly modified by smoking, although the risks were highest among nonsmokers. No effect modification by smoking was observed for the relation of BMI to esophageal squamous cell carcinoma or noncardia gastric adenocarcinoma (data not shown). In addition, no significant effect modification by history of gastroesophageal reflux disease or by educational level was found for any of the four cancer types. The findings for usual BMI were similar for diffuse and intestinal types of adenocarcinoma within subsites of stomach.

Discussion

This population-based case–control study revealed that excess weight is a strong risk factor for esophageal adenocarcinoma, with risk rising consistently with increasing BMI. The risk appeared related largely to elevated BMI *per se* and not to weight gain or loss during adult life. Furthermore, within each weight level, the risk tended to decrease with increasing height. To a lesser extent, excess weight increased the risk of gastric cardia adenocarcinoma, while no effect was seen for noncardia gastric adenocarcinoma or esophageal squamous cell carcinoma. The relatively large study size and standardized classification of case patients by study pathologists enabled us to assess the relation of anthropometric variables to the four types of esophageal and stomach cancers. These results argue against recall bias or differential reporting between case patients and control subjects, since recall or reporting of height and weight is unlikely to vary by case type.

Table 4. Odds ratios (ORs) and 95% confidence intervals (CIs) for esophageal adenocarcinoma in relation to body mass index (BMI), stratified by selected subject characteristics

	Usual BMI quartiles							
	I—low (males: <23.12; females: <21.95)		II (males: 23.12–25.08; females: 21.95–24.12)		III (males: 25.09–27.31; females: 24.13–27.43)		IV—high (males: ⩾27.32; females: ⩾27.44)	
	Case patients/ control subjects*	OR†	Case patients/ control subjects	OR (95% CI)†	Case patients/ control subjects	OR (95% CI)†	Case patients/ control subjects	OR (95% CI)†
Age group, y								
<50	2/21	1.0	5/25	5.7 (0.4–89.0)	15/15	43.5 (2.6–731.0)	13/17	33.6 (2.1–552.0)
50–59	8/40	1.0	11/37	1.7 (0.5–5.8)	13/36	3.4 (1.0–11.1)	23/41	4.5 (1.4–14.1)
60–69	12/64	1.0	17/61	1.0 (0.4–2.5)	22/63	1.5 (0.6–3.6)	32/67	2.3 (1.0–5.4)
70–79	23/47	1.0	30/50	1.3 (0.6–2.8)	35/62	1.3 (0.6–2.8)	31/48	1.7 (0.8–3.8)
Cigarette smoking								
Nonsmoker	5/49	1.0	6/51	0.6 (0.1–2.7)	16/56	4.0 (1.1–14.7)	25/51	8.7 (2.4–31.1)
Former smoker	26/69	1.0	32/67	1.5 (0.7–3.1)	38/75	1.6 (0.8–3.4)	48/84	2.1 (1.1–4.2)
Current smoker	11/47	1.0	24/41	2.0 (0.8–5.2)	25/37	2.7 (1.0–6.8)	23/29	2.9 (1.1–7.6)
GERD‡								
No	23/132	1.0	26/126	1.1 (0.5–2.2)	42/119	2.0 (1.1–3.9)	44/119	2.6 (1.4–5.0)
Yes	22/40	1.0	37/47	1.4 (0.6–3.1)	43/57	1.6 (0.8–3.5)	55/54	2.6 (1.2–5.6)
Education								
⩽High school	21/77	1.0	28/83	1.1 (0.5–2.2)	50/70	2.5 (1.3–5.0)	59/77	3.7 (1.9–7.2)
Vocational/some college	15/45	1.0	16/35	1.8 (0.6–4.9)	22/44	1.5 (0.6–4.0)	25/51	2.1 (0.8–5.4)
⩾College graduate	9/50	1.0	19/55	2.2 (0.7–7.6)	13/62	2.4 (0.7–8.0)	15/45	3.3 (1.0–11.4)

*The number of subjects do not add up to the total number interviewed because of missing values in some categories.

†Adjusted for geographic location, sex, race, respondent status, and age and cigarette smoking (when appropriate).

‡Gastroesophageal reflux disease (GERD) (severe heartburn, acid regurgitation, or dysphagia at least weekly, at least weekly use of over-the-couter antacids for at least 2 years, or ever use of H_2 blockers).

Our findings provide strong support for a causal relation between adiposity and adenocarcinomas of the esophagus and gastric cardia. Limited evidence from previous population-based, case–control studies in the United States suggested a threefold increased risk of esophageal adenocarcinoma among white men with BMI greater than 26.6 *(10)* and a 150% excess risk of esophageal adenocarcinoma as well as a 60% excess risk of gastric cardia adenocarcinoma among subjects in the highest decile of BMI *(11)*. In China, where the study population was relatively lean, the risk of gastric cardia adenocarcinoma was elevated 40% among women and threefold among men in the highest quartile of BMI *(12)*. In two studies, a dose–response relation was noted between BMI and increased risk of gastric cardia adenocarcinoma *(11,12)*. The inverse associations between height and risks of esophageal and gastric cancers also are consistent with our observation of elevated risks among the obese, since taller subjects tend to be leaner when adjusted for weight.

The mechanism by which overweight increases the risk of adenocarcinomas of the esophagus and gastric cardia is not clear. It has been suggested that obesity promotes gastroesophageal reflux disease by increasing intra-abdominal pressure *(16,17)*. In turn, gastroesophageal reflux predisposes to Barrett's esophagus, a metaplastic precursor state for adenocarcinomas of the esophagus and gastric cardia *(18,19)*. In our study, the magnitude of relative risk associated with BMI was similar among those with or without a self-reported history of gastroesophageal reflux, suggesting that obesity may influence cancer risk through mechanisms in addition to reflux *(20,21)*. However, given the relatively low sensitivity of reported symptoms for the diagnosis of gastroesophageal reflux disease *(22)*, this condition may have been substantially underreported in our study. Further investigations are needed to identify factors that may influence the cancer risks associated with obesity and gastroesophageal reflux disease, including body fat distribution, dietary practices, medications, and other conditions that may affect the frequency and severity of reflux disease and the composition of enterogastric refluxate.

If the association with usual BMI is causal and our relative risk estimates reflect the true magnitude of associations, attributable risk calculations indicate that individuals above the median level of BMI may account for 33% of esophageal adenocarcinoma and 22% of gastric cardia adenocarcinoma case patients occurring in the three geographic areas over the study period. Therefore, the upward trend in the incidence of these tumors may be related in part to substantial increases in the prevalence of overweight in the U.S. population. The prevalence of overweight adults [defined in *(23)* as BMI $\geqslant$27.8 for men and $\geqslant$27.3 for women, which are approximately equal to the highest quartiles of usual BMI in our study] at all ages rose from 25% in 1976 through 1980 to 33% in 1988 through 1991, with the greatest increase occurring among men age 50 years or older *(23)*.

The only environmental factor consistently linked to adenocarcinomas of the esophagus and gastric cardia in epidemiologic studies has been cigarette smoking *(9,11,24–27)*. In our study, increases in risk with usual BMI were greatest among nonsmokers, indicating that smoking is not a necessary cofactor for the association with overweight. Our finding that the BMI-associated risk is highest in the youngest age group suggests that obesity is particularly important for early-onset tumors, while other risk factors may assume a more prominent role for tumors developing in later years.

It is unclear why risks associated with overweight were greater for esophageal adenocarcinoma than for gastric cardia adenocarcinoma. It is possible, although unlikely, that some noncardia gastric cancers may be misclassified as cardia tumors, despite our standardized review and classification procedures, thus attenuating the association of gastric cardia adenocarcinoma with overweight. It is also possible that reflux mechanisms are more closely related to Barrett's esophagus and subsequent esophageal adenocarcinoma than to the development of gastric cardia cancer. In contrast, we found no association between BMI and noncardia gastric cancer and a slight but nonsignificant inverse relation with squamous cell carcinoma of the esophagus. The latter finding is consistent with the inverse correlation between body weight and the major risk factors (smoking, drinking, and poor nutrition) for squamous cell esophageal cancer *(28)*, although additional adjustment for alcohol drinking or caloric intake did not affect our results.

In summary, our multicenter population-based, case–control study found that increased BMI was a strong risk factor for esophageal adenocarcinoma and a moderate risk factor for gastric cardia adenocarcinoma. The elevated risks appeared related mainly to excess weight *per se* and not to weight changes over time. In contrast, BMI was largely unrelated to esophageal squamous cell carcinoma and noncardia gastric adenocarcinoma. These findings suggest that the increasing prevalence of obesity in the population has contributed to the rising incidence trends for adenocarcinomas of the esophagus and gastric cardia. Further epidemiologic, clinical, and laboratory studies are needed to identify the mechanisms by which obesity increases the risk of these tumors.

References

(1) Yang PC, Davis S. Incidence of cancer of the esophagus in the US by histologic type. Cancer 1988; 61:612–7.

(2) Blot WJ, Devesa SS, Kneller RW, Fraumeni JF Jr. Rising incidence of adenocarcinoma of the esophagus and gastric cardia. JAMA 1991;265: 1287–9.

(3) Powell J, McConkey CC. The rising trend in oesophageal adenocarcinoma and gastric cardia. Eur J Cancer Prev 1992;1:265–9.

(4) Hansson LE, Sparen P, Nyren O. Increasing incidence of both major histological types of esophageal carcinomas among men in Sweden. Int J Cancer 1993;54:402–7.

(5) Blot WJ, Devesa SS, Fraumeni JF Jr. Continuing climb in rates of esophageal adenocarcinoma: an update [letter]. JAMA 1993;270: 1320.

(6) Yang PC, Davis S. Epidemiological characteristics of adenocarcinoma of the gastric cardia and distal stomach in the United States, 1973–1982. Int J Epidemiol 1988;17:293–7.

(7) Powell J, McConkey CC. Increasing incidence of adenocarcinoma of the gastric cardia and adjacent sites. Br J Cancer 1990;62:440–3.

(8) Zheng T, Mayne ST, Holford TR, Boyle P, Liu W, Chen Y, et al. The time trend and age-period-cohort effects on incidence of adenocarcinoma of the stomach in Connecticut from 1955–1989. Cancer 1993;72:330–40.

(9) Gammon MD, Schoenberg JB, Ahsan H, Risch HA, Vaughan TL, Chow WH, et al. Tobacco, alcohol, and socioeconomic status and adenocarcinomas of the esophagus and gastric cardia. J Natl Cancer Inst 1997;89:1277–84.

(10) Brown LM, Swanson CA, Gridley G, Swanson GM, Schoenberg JB, Greenberg RS, et al. Adenocarcinoma of the esophagus: role of obesity and diet. J Natl Cancer Inst 1995;87:104–9.

(11) Vaughan TL, Davis S, Kristal A, Thomas DB. Obesity, alcohol, and tobacco as risk factors for cancers of the esophagus and gastric cardia:

adenocarcinoma versus squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev 1995;4:85–92.

(12) Ji BT, Chow WH, Yang G, McLaughlin JK, Gao RN, Zheng W, et al. Body mass index and the risk of cancers of the gastric cardia and distal stomach in Shanghai, China. Cancer Epidemiol Biomarkers Prev 1997;6:481–5.

(13) Waksberg J. Sampling methods for random digit dialing. J Am Stat Assoc 1978;73:40–6.

(14) Hatten J. Medicare′s common denominator: the covered population. Health Care Finance Rev 1980;2:53–64.

(15) Breslow NE, Day NE. Statistical methods in cancer research. Volume I—The analysis of case-control studies. IARC Sci Publ 1980;32: 5–338.

(16) Hogan WJ, Dodds WJ. Gastroesophageal reflux disease (reflux esophagitis). In: Sleisenger MH, Fordtran JS, editors. Gastrointestinal disease. 4th ed. Philadelphia: Saunders, 1989: 594–619.

(17) Day JP, Richter JE. Medical and surgical conditions predisposing to gastroesophageal reflux disease. Gastroenterol Clin North Am 1990;19: 587–607.

(18) Chow WH, Finkle WD, McLaughlin JK, Frankl H, Ziel HK, Fraumeni JF Jr. The relation of gastroesophageal reflux disease and its treatment to adenocarcinomas of the esophagus and gastric cardia. JAMA 1995;274:474–7.

(19) Reid BJ, Barrett MT, Galipeau PC, Sanchez CA, Neshat K, Cowan DS, et al. Barrett's esophagus: ordering the events that lead to cancer. Eur J Cancer Prev 1996;5 (Suppl 2):57–65.

(20) Castell DO. Obesity and gastro-oesophageal reflux: is there a relationship? Eur J Gastroenterol Hepatol 1996;8:625–6.

(21) Mathus-Vliegen LMH, Tytgat GNJ. Twenty-four-hour pH measurements in morbid obesity: effects of massive overweight, weight loss and gastric distension. Eur J Gastroenterol Hepatol 1996;8:635–40.

(22) Klauser AG, Schindlbeck NE, Muller-Lissner SA. Symptoms in gastro-oesophageal reflux disease. Lancet 1990;335:205–8.

(23) Kuczmarski RJ, Flegal KM, Campbell SM, Johnson CL. Increasing prevalence of overweight among US adults. The National Health and Nutrition Examination Surveys, 1960 to 1991. JAMA 1994;272:205–11.

(24) Kabat GC, Ng SK, Wynder EL. Tobacco, alcohol intake, and diet in relation to adenocarcinoma of the esophagus and gastric cardia. Cancer Causes Control 1993;4:123–32.

(25) Brown LM, Silverman DT, Pottern LM, Schoenberg JB, Greenberg RS, Swanson GM, et al. Adenocarcinoma of the esophagus and esophagogastric junction in white men in the United States: alcohol, tobacco, and socioeconomic factors. Cancer Causes Control 1994;5:333–40.

(26) Ji BT, Chow WH, Yang G, McLaughlin JK, Gao RN, Zheng W, et al. The influence of cigarette smoking, alcohol, and green tea consumption on the risk of carcinoma of the cardia and distal stomach in Shanghai, China. Cancer 1996;77:2449–57.

(27) Zhang ZF, Kurtz RC, Sun M, Karpeh M Jr, Yu GP, Gargon N, et al. Adenocarcinomas of the esophagus and gastric cardia: medical conditions, tobacco, alcohol, and socioeconomic factors. Cancer Epidemiol Biomarkers Prev 1996; 5:761–8.

(28) Munoz N, Day NE. Esophageal cancer. In: Schottenfeld D, Fraumeni JF Jr., editors. Cancer epidemiology and prevention. 2nd ed. New York: Oxford University Press, 1996:681–706.

Notes

Present address: A. B. West, Department of Pathology, The University of Texas, Galveston.

Supported in part by Public Health Service grants CA57983, CA57949, and CA57923 and by contracts CP40501 and CN05230 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

We thank the study managers, Sarah Greene and Linda Lannom, and the field supervisors, Patricia Owens, Tom English, and Berta Nicol-Blades, for their dedicated efforts in data collection and processing and the staff of 178 hospitals in Connecticut, New Jersey, and Washington for their participation in the study.

Manuscript received July 1, 1997; revised October 29, 1997; accepted November 14, 1997.